NANOTECHNOLOGY CHARACTERIZATION LABORATORY

NCL Method STE-2
Version 1.0

Detection of Microbial Contamination Assay

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This protocol assumes an intermediate level of scientific competency with regard to techniques, instrumentation, and safety procedures. Rudimentary assay details have been omitted for the sake of brevity.

Method is written by: _____ 9 Mar 06.

Marina A. Dobrovolskaia, Ph.D. Date

Testing facility: NCL, NCI-Frederick, Bldg 469, Room 250.

1. Introduction

This protocol describes a procedure for quantitative determination of microbial contamination in a nanoparticle preparation. The protocol includes tests for yeast, mold and bacteria using Millipore sampler devices and is based on Millipore Samplers, dilution kits and swab test kits user guide. The assay requires 1.0 mg of test nanomaterial.

2. Reagents

2.1. Sterile PBS, Sigma, cat # D8537

2.2. Yeast and mold sampler, Millipore Corp., cat# MY0010025

2.3. Total count sampler, Millipore Corp., cat# MT0010025

2.4. HPC Count sampler, Millipore Corp., cat# MHPC10025

2.5. Test nanomaterial

2.6. Buffer used to reconstitute test nanomaterial

2.7. Sodium Hydroxide, Sigma, cat.#S2770

2.8. Hydrochloric acid, Sigma H9892

Note: Equivalent reagents from other vendors can be used

3. Equipment

3.1. Pipettes covering range from 0.05 to 1 mL

3.2. Sterile tubes 5 mL

3.3. Vortex

3.4. Pipet tips 0.5 μL – 1.0 mL

3.5. Sterile pipets, 1-10 mL

3.6. Incubator, set at 35°C

4. Reagent Preparation

4.1. <u>Sodium Hydroxide</u>

Prepare from concentrated stock by dilution into sterile water to make solution with final concentration of 0.1N.

4.2. <u>Hydrochloric Acid</u>

Prepare from concentrated stock by dilution into sterile water to make solution with final concentration of 0.1N.

5. Preparation of Quality Controls

Use sterile PBS or water as negative control. Negative control is acceptable if no CFU is observed upon completion of the test. For the positive control use bacterial or yeast cell cultures (ATCC #25254 and MYA774 , respectively) at dilution to allow at least 10 CFU/mL. If standard cultures are not available, a sample from other source (e.g. rain water, floor swipe etc) known to contain bacteria and yeast/mold may be used. A positive control is acceptable if it allows identification of at least 10 CFU/mL.

6. Preparation of Study Samples

Test nanoparticles should be reconstituted in sterile PBS to a final concentration of 1 mg/mL. The pH of the study sample is checked using pH microelectrode and is adjusted with sterile either NaOH or HCl as necessary to be within pH range 6-8. If NaOH or HCl is not compatible with a given nanoparticles formulation, adjust pH using a procedure recommended by nanomaterial manufacturer. To avoid sample contamination from microelectrode, always collect a small aliquot of the sample and use it to measure the pH.

7. Experimental Procedure

7.1. Remove the Sampler from its plastic bag and write on the case with indelible marker the date, and the sample reference number.

7.2. Using sterile conditions remove a paddle from case and apply 1 mL of nanoparticle preparation or dilution onto the surface of a filter. Allow liquid to absorb, then recap the paddle. To prevent the paddle from drying out during incubation, it should be seated firmly in the case to form air-tight seal.

7.3. Incubate for 72h at a nominal temperature of 35°C.

7.4. Remove paddle from case and examine for appearance of colonies. Perform colonies count.

7.5. Report results according to the following formula: # colonies x dilution = CFU/mL

8. Acceptance Criteria

8.1. The test result is acceptable if positive and negative controls are acceptable.

8.2. Test nanomaterial is acceptable if it demonstrated negative results.

9. References

9.1. Samplers, Dilution kits and swab test kits user guide. P15325, Rev. D., 8/99 Millipore Corp.